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Measurement of Oral Malodor: Current Methods and Future Prospects

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MEASUREMENT OF ORAL MALODOR is complicated by a variety of parameters including complexity of gaseous molecular species, sampling difficulties, temporal variations, choice of suitable subject populations, and lack of agreement on reference standards. Since oral malodor is a perceived olfactory stimulus, direct sampling and assessment by human judges may be the most logical measurement approach. However, as with other psychophysical assessments, human malodor measurement by the human nose may vary widely among and between judges, and consequently cannot be confidently reproduced in other laboratories. Such shortcomings have led several investigators to propose quantitative approaches based on measurement of volatile sulfide compounds which are associated with oral malodor. Highly sensitive and discriminatory measurements of volatile sulfides can be made using gas chromatography although for rapid sampling of larger subject populations, portable sulfide monitors may be more appropriate. Future research in this field should consider: 1) improved and simplified instrumentation for more rapid throughput and reliability; 2) development and definition of reference standards for oral malodor assessment; 3) formulation of clinical studies with appropriately sized, well-defined patient populations; and 4) further development of within mouth, site-specific measurements. *J Periodontol 1992; 63:776–782.*

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Oral malodor (i.e., bad breath, halitosis, *fetor ex ore*) is a common complaint that may periodically affect most of the adult population.[1,2] In the large majority of cases, oral malodor originates in the oral cavity as the result of microbial metabolism.[1,3–5] Malodor of oral etiology may result from microbial metabolism on the tongue dorsum and periodontium, and is modulated by low salivary flow at certain times during the day, food impaction, and diet.[6–12] Non-oral etiologies may include upper and lower respiratory tract conditions, neurologic and gastrointestinal disorders, various systemic diseases, and use of certain drugs.[13]

Oral malodor is a subject of considerable public interest, yet has engendered only limited scientific investigation in part because of the difficulties of measurement. Thus, although a circle of acquaintances might generally agree that a certain person suffers from bad breath, scientific assessment and validation of oral malodor measurements have not been realized. The purpose of this manuscript is to review direct and indirect techniques for measuring malodor, to discuss the problems associated with selecting subjects and sampling, and to raise some prospects for future research in this area.

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Subjective Measurement of Oral Malodor

The most simple and commonly used approach to sample and measure oral malodor is direct nasal sniffing of expelled mouth air. This is often referred to as "organoleptic" or "hedonic" assessment. In a sense, this method may be considered a kind of reference standard of oral malodor measurement. This approach closely simulates the everyday situations in which bad breath is detected. However, such organoleptic measurements also raise several problems.

One concern is that there is considerable variation between judges on the ranking of the unpleasantness of different odors.[14–18] Thus it is not surprising that appraisal of bad breath differs from one judge to another.[18] Furthermore, a wide variety of confounding factors including psychological/physiological states (e.g., hunger, menstrual cycle, head position, degree of attentiveness and expectation[14–16]) influence organoleptic measurements. Moreover, olfactory sampling is best performed by taking a short, rapid sniff,[14] an activity which is difficult to perform repeatedly in exactly the same fashion.

To reduce inter-examiner variations, a panel consisting of several judges is often employed, and the level of malodor is

based on the mean score.[1,7,11,12,18–21] This procedure however, leads to sampling problems. Since it is not feasible for several judges to measure the bad breath of a given subject simultaneously, sequential measurements are often made.[7] This method introduces additional complications; once the oral headspace is sampled for measurement by one judge (e.g., by having the subject breathe out through the mouth), it is unlikely that the concentration and composition of the various gases are reproduced in subsequent samples.

Olfactory assessment of odor is one example of psychophysical measurements that include other sensations such as pain and taste. Such parameters are difficult to quantify, and bad breath is no exception. The intensity of oral malodor observed by human judges is usually graded on a scale consisting of three or more descriptive levels (e.g., no odor, weak, middle, or strong); optimally, the odor judge treats these categories as if separated from one another by equivalent intervals.[18,20,22–24] One of the drawbacks of such scoring is that it is often difficult to decide on intermediate grades (e.g., whether a given sample should be scored "2" or "3" on a scale of zero to 5). Moreover, whereas the zero score usually represents no observable odor, any top score (e.g., extremely foul) is open-ended, and may cover a wide distribution of extremely foul odors. Apparently, no studies have attempted to use continuous scales, which have been introduced in other areas of psychophysics. In early studies of malodor, an osmoscope was used in conjunction with a scale based on the highest dilution at which malodor could be detected.[8,25,26] For reasons that are not clear, linear increases in organoleptic ratings usually correspond to exponential (rather than linear) increments in odorant concentration.[14,24]

Should judges of oral malodor receive prior training or undergo precalibration procedures? The reliability and reproducibility of organoleptic measurements may possibly be improved if the judges are trained to recognize and rate oral malodor. In a recent study comparing an experienced and a novice oral malodor judge, odor scores of the former were more reproducible, and gave higher correlations with instrumental sulfide analysis.[19] Furthermore, agreement among panel judges might be higher if they are previously instructed to assign certain ratings to given levels of odor stimuli.[14–17] However, reproducible "bad breath standards" do not exist, thus a preview of assigned stimuli prior to the experiment [24] is not feasible. Furthermore, training and precalibration procedures might limit individual judgment, thus biasing scoring towards that of the instructor. In one study in which 7 untrained judges independently scored the oral malodor of 75 subjects, inter-examiner correlation coefficients (Spearman) were significant but not high, ranging from 0.14 ($P = 0.14$) to 0.49 ($P < 0.001$). Interestingly, 2 of the judges' ratings appeared to correlate poorly with the other 5 judges, yet correlated with one another to a higher degree.[18] This suggests that certain individuals may respond differently to various components of oral malodor.

While general olfactory tests are available for selecting odor judges,[15,16] little information is available on how judges for bad breath assessment should be selected or excluded. First, since odor perception is purportedly more developed in females than males, it is conceivable that females may be better judges than males.[27] Second, there is a decrease in the ability to perceive odors with increasing age.[27] Third, if people become adapted to their own oral malodor, should potential judges be excluded if their own malodor exceeds a predetermined level? In this context it should be recognized that in the clinical setting, it is the dental professionals who should notice and treat oral malodor. Yet if dentists become habituated to oral malodor just as workers in other foul-smelling surroundings (e.g., tanneries) become accustomed to workplace odors,[14] dentists may have a reduced ability to recognize and assess oral malodor.

Instrumental Analysis of Oral Malodor

Over the past three decades, Tonzetich and coworkers have developed instrumental analysis of oral malodor using gas chromatography (GC) coupled with flame photometric detection.[1,11,22,27–31] These investigations have led to the conclusion that oral malodor is associated primarily with volatile sulphur compounds (VSC). Tonzetich proposed that among the sulphur gases, hydrogen sulfide and methyl mercaptan were the main odor-causing components. Recently, Yaegaki and coworkers have shown that hydrogen sulfide is produced largely from the tongue dorsum, and that malodor from periodontal tissues is enriched with methyl mercaptan and dimethyl disulfide.[12] GC measurement has been used to demonstrate malodor reduction after use of mouthrinses, as well as mechanical oral hygiene techniques.[20–23]

Gas chromatographic measurement of malodor samples has several advantages compared to organoleptic measurement including: 1) separation and quantitative measurement of individual gases; and 2) the ability to measure extremely low concentrations of gases. The main disadvantages of such instrumentation are: 1) relatively high cost; 2) the need for skilled personnel; 3) cumbersome and lack of portability; and 4) the time required for detection and measurement (sample throughput). As a result of the labor-intensive nature of GC sample assessment, many malodor investigations have involved relatively small numbers of subjects. For example, the clinical studies of Tonzetich and coworkers,[11,22,30,31] Solis-Gafar et al.,[32] and Kostelc and coworkers,[6,33,34,37] employed 20, 8, 15, 5, 13, 10, 14, 23, and 17 subjects, respectively (see also Table 1).

There are few published studies on the correlation between organoleptic ratings and the level of VSC as determined by GC techniques. Unfortunately, studies which employed both methods to test the efficacy of various treatments did not provide statistical comparisons of the measurement techniques themselves. In one of the few studies which presented such data, Schmidt et al.[20] compared GC with organoleptic ratings by three judges using a scale of 0 to 3. Thirty-six subjects (18 men and 18 women) who

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Table 1: Summary of Some Clinical Oral Malodor Studies

Subject Population (Reference)	Measurement Techniques	Main Findings
5 matched pairs of healthy students, one group abandoning oral hygiene for 30 days (6)	GC-flame photometric analysis of mouth air; GC-mass spectrometric analysis of saliva headspace	Increases in breath and salivary volatiles in experimental group
75 adults (18)	Peak sulphide measurements, using portable monitor, vs. mean of 7 organoleptic judges (both measurements of whole mouth odor)	Significant ($P < 0.001$) correlation ($r = 0.60$) between the 2 methods
41 adults complaining of bad breath (19)	Organoleptic measurements of whole mouth, tongue and floss, sulphide measurements with portable monitor	Steady-state sulfide measurements were most reproducible
102 adult subjects in two separate studies (20)	GC-flame photometric analysis of VSC in mouth air, vs. organoleptic whole mouth measurement by 3 professionally-trained odor judges	Significant correlations between organoleptic results and total hydrogen sulfide + methyl mercaptan
8 male non-smokers with objectionable early morning mouth oder (20)	GC-flame photometric analysis and 8-member organoleptic panel measurement of whole mouth odor	Reductions in sulfides and malodor following oral hygiene procedures
30 adult subjects with over 30 ppb of methyl mercaptan in their early morning mouth air (23)	GC mouth odor analysis; organoleptic analysis by 5 judges through glass tubes, through holes in a partition; microbial sampling for odorigenic microorganisms	Decreases in all measurements following mouthrinsing with active mouthrinse, vs. placebo rinse and water
100 patients with periodontal problems vs. 100 with normal periodontia (26)	Paraffin-stimulated saliva samples with added casein were analyzed for sulphides and indole, following 1 and 3 hours incubation	Saliva from pathological cases putrefied more rapidly than from normal cases, producing higher levels of sulfides and indole.
15 non-smokers, 10–50 years old (30)	GC-flame photometric analysis of VSC in mouth air and putrefied saliva headspace	Suitable system for detecting VSC and measuring treatment effects
5 healthy females 21–30 years old (31)	GC analysis of hydrogen sulphide, methyl mercaptan, and dimethylsulphide in mouth air	Cyclical changes in VSC during menstrual cycle
13 adult males, non-smokers, with good oral health, but high levels of VSC in morning breath (32)	GC-flame photometric analysis of mouth air, of subjects rinsing with either placebo or active mouthrinse	Reductions in hydrogen sulfide (14%) and methyl mercaptan (45%) in the mouthrinse group
7 healthy subjects vs. 7 with periodontitis (33)	GC and GC-mass spectrometry of putrefied saliva headspace; note difference in collection of saliva between the 2 groups	Pyridine and methyl pyridines (picolines) found only in diseased group
13 "normal" vs. 10 with "moderate or advanced periodontal involvement" (34)	GC and GC-mass spectrometry of putrefied saliva headspace	Pyridines and methyl pyridines (picolines) primarily in periodontal group
17 subjects (3 females, 14 males) (37)	GC-mass spectrometry of saliva headspace	Various components of exogenous origin (pollutants, foods, cosmetics) were identified

did not exhibit any overt oral disease other than mild gingival inflammation or dental caries were studied in the first experiment; the second experiment was extended to include 66 subjects (36 males and 30 females). Whereas the correlation coefficients between VSC (hydrogen sulfide + methyl mercaptan) levels and organoleptic scores were low in both experiments (0.28 and 0.35, respectively, using Kendall rank correlation analysis), in the second experiment, the null hypothesis (i.e., no correlation between VSC and organoleptic scores) could be excluded at a higher level of confidence ($P < 0.001$). The extent of variation among the individual judges was not reported.

Recently, the application of an industrial sulfide monitor for measuring gases associated with oral malodor has been reported.[18] In an initial study, highly significant correlations were demonstrated between monitor measurements of 75 subjects, and organoleptic ratings by 7 judges, with an overall Spearman correlation of $r = 0.60$ for log-transformed sulfide scores vs. the mean of the panel of judges.[18] Similar correlations between sulfide concentrations obtained with the monitor and organoleptic ratings of odor were found in subsequent studies.[19,35] Measurements using the monitor have been shown to be more reproducible than organoleptic measurements, and more sensitive to reductions in malodor brought about by mouthrinsing.[19,35] The advantages of the monitor compared to GC include: 1) lower cost; 2) can be operated by non-skilled personnel; 3) portability; and 4) rapid turn-around time between measurements. A further possible advantage is the manner in which the sample is obtained (vide infra). The main disadvantages of the instrument in its present configuration are: 1) its inability to distinguish between individual sulfides; 2) measurement cannot be made in the presence of high levels of ethanol or essential oils, thus precluding assessment of mouthwash efficacy until these components have dissipated; and 3) the instrument may show a slight loss of sensitivity with time, necessitating periodic recalibration.[19] Studies which compare GC and sulfide monitor measurements have not been published to date, but may prove highly instructive. One major difference between the two techniques is the manner of sampling, to be discussed in a later section.

Should Malodor Originating in the Mouth Be Considered a Single Entity?

Both hydrogen sulfide and methyl mercaptan are highly volatile gases which are readily detected by GC. Nevertheless, it is possible that other odorants may be important as well. Whereas the correlations between both VSC and organoleptic measurements show that the two are, at least to

some extent, associated with one another,[18–20] it does not necessarily follow that they are chiefly responsible for oral malodor. This point has been demonstrated in the food industry, in which certain gases have been correlated with poor tasting products yet are not themselves directly responsible for the poor taste.[16] Tonzetich and coworkers sniffed the eluent of the GC and found that hydrogen sulfide and methyl mercaptan were the main malodorous components eluted.[36] However, less volatile components which evade detection might also be involved. Several observations support this view: 1) following use of antiseptic mouthrinses or oral hygiene methods, reductions in both VSC and organoleptic ratings are observed, but the reductions of VSC are much more dramatic than the reductions of organoleptic scores;[11,21–23] 2) bad breath is more noticeable when the mouth dries out (e.g., following fasting,[30] a long period of talking, or sleeping[1,11]), suggesting that malodorous molecules are retained in or beneath the water film covering the oral mucosa but, following drying of the tissue, can be volatilized; 3) despite the possibility that the mixture of oral gases changes the nature of the odor of the individual components, neither hydrogen sulfide nor methyl mercaptan smells like bad breath; and 4) most natural odors owe their characteristic smell to tens or possibly hundreds of volatile component molecules.[14]

To illustrate the potential complexity of the oral gaseous species involved, Kostelc and coworkers found elevated levels of a variety of volatiles in the saliva headspace of subjects with periodontal disease, including dodecanol, tetradecanol, phenol, p-cresol, pyridine, and pyridine, 2-,3-,4-methylpyridine.[33,34] Whereas Tonzetich and coworkers ruled out a possible role for various components based on low volatility, these experiments were based on their release from liquid (saliva) to gas (air), and may not reflect their volatility on dry oral surfaces.

If a number of odorants are involved in malodor of oral etiology, it is worth considering the possibility that their relative levels vary among individuals. For example, there is usually an appreciable difference between odor originating from the tongue and that emanating from dental floss passed interproximally.[19] Yaegaki and Sanada[12] have provided evidence that the hydrogen sulfide/methyl mercaptan ratio in tongue and oral malodor changes as a function of the severity of periodontal disease, although it is unclear whether variations in the concentrations of these two gases can account for the difference in the qualities of odor observed.

Sample Populations

The choice of participants in a clinical study of oral malodor is an important, but largely ignored, aspect of oral malodor research and may be crucial to our understanding of the nature of oral malodor and the homogeneity of the data obtained. For example, the overall contribution of malodor from the tongue dorsum may differ in healthy subjects vs. those with periodontal diseases.[12] One potential approach to improve the homogeneity of the study population would be to include only subjects who think they have bad breath. However, as recently shown, a large proportion of such subjects who are convinced that they suffer from oral malodor do not necessarily present with intense malodor levels.[19]

After deciding upon inclusion criteria, the choice of exclusion criteria may also improve the homogeneity of the data set and thus permit more clear-cut conclusions. For example, because of fluctuations in malodor associated with the menstrual cycle, women may make less suitable subjects than men.[31] Exclusion criteria should also consider smoking, dietary factors (e.g., onions and garlic), medication, time of year, and dental treatment. Exclusion criteria may reduce data variation of the results obtained, but also restrict the findings to the subclasses of subjects examined.

Obtaining the Sample

Bad breath may be compared to other cases of pollution of the ambient atmosphere emanating from confined sources. Points of discharge from confined sources (e.g., smokestacks) have characteristic moisture contents, temperature, shape, discharge rates, dilution, and dispersion variables.[38] Awareness of bad breath comes only after release from the mouth into the open environment. In both cases, the physical conditions at the site of sampling are far different from those at the origin. Thus the method of obtaining the sample itself contributes to bias and variation.

We usually notice bad breath while someone is talking to us. Yet, measurement in such a manner has not been reported. In GC studies, the sample is usually obtained by drawing air from the headspace within the mouth (e.g., with a syringe). Conversely, in subjective organoleptic panel measurements, the subject is instructed to breathe out through the mouth. Both these approaches have drawbacks. In a closed mouth, a concentration gradient probably exists, with the highest levels of odorants proximal to the surfaces from which they emanate. When the mouth is opened for headspace sampling, turbulence may contribute to unrepresentative sampling of mixtures of gases when the syringe sample is drawn and may result in a completely different mix of gases than that obtained by static sampling. In organoleptic measurements, the mouth air is largely diluted by lung air as the subject rapidly breathes out. Neither of these methods replicates the flow of malodor outwards from the mouth during speech, for example. One potential solution to this problem is to blow air into the mouth and measure the effluent emitted from the mouth, having the patient refrain from mouthbreathing during measurement.[26] A similar approach is employed with the portable sulfide monitor, which draws air out of the semi-open oral cavity at a fixed rate while the subject breathes through the nose.[18] Initially, a peak sulfide measurement is observed, which reflects the standing headspace level. Subsequently, as the oral cavity is washed by the continual stream of air sucked into and

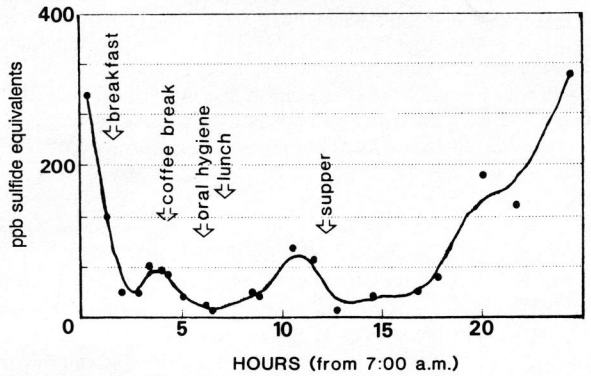

Figure 1. Oral malodor as a function of time of day. Volatile sulfides, in peak ppb hydrogen sulfide equivalents, were measured in a single subject (MR), over the course of a day, starting at 7:00 a.m. Measurements were made using a 1170 portable sulfide monitor as described in reference 19. Significant oral activities are indicated.

from the mouth, the measurement falls to a steady state level. This parameter apparently reflects the contributions of ongoing malodor production, together with mass transfer of odorants from the oral surfaces into the gaseous phase. Indeed, such steady state values proved more reproducible than the initial peak measurements obtained.[19]

Finally, the temporal aspects and frequency of sampling cannot be overlooked. The level of oral malodor in an individual may vary greatly over the course of a single day (Fig. 1). Nevertheless, if subjects are required to refrain from potentially confounding oral activities for several hours prior to examination, reproducible measurements can be made a week apart.[19]

Whole Mouth Versus Site Specific Measurement

It should be emphasized that the measurements described above assess whole mouth malodor, whereas it is likely that the origins of the malodor are localized (e.g., foci on the tongue dorsum, or specific periodontal pockets). Rizzo detected the presence of hydrogen sulfide in individual pockets using paper impregnated with lead acetate.[39] Refinement of such an approach, which would yield quantitative data on malodor production at various oral sites might provide information on metabolism at specific sites in the periodontium and could over the long-term lead to an improved understanding of fluctuations in periodontal disease activity and perhaps other oral diseases (e.g., white lesions of oral mucous membranes, candidiasis). Rosenberg et al.[19] attempted to pinpoint loci of malodor by organoleptic assessment of floss following interproximal passages, as well as gauze rubbed along the tongue. Although there was some statistical association between these parameters and whole mouth assessments, neither of the two techniques proved reliable.

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Indirect Measurement of Oral Malodor

The variability of oral malodor measurements and the difficulties in assessing oral malodor directly from the oral cavity have led investigators to employ indirect methods. The approach which has been most widely employed is the measurement of malodor and volatile sulfides in putrefying saliva samples.[1,6,11,12,28–31,36,37,40] In most cases, saliva obtained directly from the mouth possesses little or no foul odor, but after several hours of in vitro incubation, malodor is easily detected, due primarily to breakdown of particulate matter by microorganisms.[12] Whereas some extrapolations between the odor of putrefied saliva and oral malodor may be acceptable, caution should be exercised since the odor of putrefied saliva usually differs appreciably from directly-sampled oral malodor. A second indirect method which has been less frequently employed is the measurement of sulphides and malodor from bacterial isolates and pure cultures.[3–5,23]

Self-Assessment of Oral Malodor

Considering the many hundreds of millions of dollars that are spent annually on mouthwashes, sprays, candies, and chewing gums that are marketed on the basis of breath freshening, it is evident that oral malodor is of concern to a substantial proportion of the North American population. Few individuals seek or receive adequate professional counseling.[41] It has usually been assumed that we do not notice our own oral malodor, presumably because we become adapted (habituated) to it.[1,2,8,38] Thus, people possessing foul bad breath are often unaware of it, whereas many individuals who have relatively low malodor levels may be excessively concerned that they have bad breath. Clearly, self-diagnosis of bad breath is a subject of considerable public interest. There are at least three methods by which people can attempt to diagnose their own oral malodor. Firstly, one may cup one's hands over the mouth and nose, breathe out through the mouth and immediately take a short sniff. In many situations (especially upon arising) malodor may be perceived. Of course, it is difficult to ascertain whether such a self-measurement provides an objective estimation, but nevertheless it may be considered as the minimal level which others might notice. Secondly, patients may be alerted to the foul odor which often accompanies dental floss following interproximal passage. Such odors probably make a significant contribution to whole mouth odor. Finally, one's tongue odor may be conveniently assessed by extending the tongue out as far as possible, licking one's wrist, waiting for several seconds, and then smelling. Although these last two approaches are indirect, they may be of diagnostic and therapeutic value, and are currently under experimental evaluation.

Future Prospects

Oral malodor is a subject of considerable public interest yet, as described above, scientific advances have been stymied by the lack of simplified instrumentation and the need for

detailed molecular studies that dissect the component species of oral gases. Progress in the future will rely on: 1) improved instrumentation with the capacity for multiple species sampling and with high patient throughput; 2) development and general agreement on reference standards of oral malodor gaseous species so that interlaboratory comparisons can be made; 3) design and conduct of clinical studies with appropriate inclusion/exclusion criteria, sample size, and outcome variables; 4) development of site-specific sampling methods within the mouth; and 5) association studies of the relationship between oral malodor and significant oral diseases.

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